

**ABSTRACT**

In this application is described substrates for  
5 high-throughput assays of clostridial neurotoxin  
proteolytic activities. Two types of substrates are  
described for use in assays for the proteolytic  
activities of clostridial neurotoxins: (1) modified  
peptides or proteins that can serve as FRET substrates  
10 and (2) modified peptides or proteins that can serve  
as immobilized substrates. In both types a  
fluorescent molecule is present in the substrate,  
eliminating the requirement for the addition of a  
fluorogenic reagent. The assays described can be  
15 readily adapted for use in automated or robotic  
systems.